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N-Desmethylclobazam: a possible alternative to clobazam in the treatment of refractory epilepsy?

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1 The development of anticonvulsant tolerance during 10 days treatment with either clobazam or its principal metabolite, *N*-desmethylclobazam (NDMC), was compared in mice using an i.v. infusion of pentylenetetrazole as the convulsive stimulus. Subsequently the anticonvulsant activity of NDMC was assessed in patients with refractory epilepsy.
2 In mice, a highly significant tolerance ($P < 0.001$) developed to clobazam (10 mg kg^{-1} twice daily). During the same period, there was no significant change ($P > 0.05$) in the protection afforded by NDMC (40 or 80 mg kg^{-1} twice daily) although some reduction in anticonvulsant activity was apparent.
3 NDMC (30 mg once daily) was given to nine patients with frequent complex partial and/or grand mal seizures who had become tolerant to the anticonvulsant effect of clobazam. Seven of the patients had been free from benzodiazepine therapy for at least 2 weeks, while the other two patients were switched directly from clobazam.
4 Eight of the nine patients showed a favourable response to NDMC. In the seven who had been given a holiday from clobazam the response to NDMC was similar to the initial response to clobazam and was achieved at plasma NDMC concentrations in the same range as those seen during clobazam administration (1000–3000 ng ml^{-1}).
5 It is concluded that NDMC is active as an anticonvulsant in man and there is evidence from the animal studies to suggest that it may be preferable to clobazam.

Keywords *N*-desmethylclobazam anticonvulsant clobazam tolerance

Introduction

The effectiveness of the 1,5-benzodiazepine, clobazam, as adjunctive therapy for refractory epilepsy is limited in many cases by the rapid development of a partial tolerance (Gastaut & Low, 1979; Allen *et al.*, 1985). This phenomenon is common to other benzodiazepines (Browne & Penry, 1973; Browne, 1976) and is the major drawback to their long-term use in the management of epilepsy. Anticonvulsant tolerance to clobazam has also been demonstrated in mice (Gent & Haigh, 1983; Gent *et al.*, 1984) and has characteristics which closely parallel many of the clinical observations. Further evidence from studies in this animal model confirms a clinical suspicion that there are important differences in the tolerance-inducing potential of various benzodiazepines (Gent *et al.*, 1985); this has stimulated an interest in finding an alternative to clobazam.

In animal studies *N*-desmethylclobazam (NDMC), the major metabolite of clobazam, is also anticonvulsant (Meldrum & Croucher, 1982; Haigh *et al.*, 1984). These results, as well as measurements of plasma clobazam and NDMC

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levels after clobazam administration in epilepsy (Callaghan & Goggin, 1984; Jawad *et al.*, 1984), suggest that NDMC is responsible for a substantial part of the antiepileptic response to clobazam. In view of this, NDMC itself would seem to merit investigation as a treatment for epilepsy.

In these experiments we have compared the tolerance induced by clobazam and NDMC in mice, and subsequently made a preliminary evaluation of the activity of NDMC in patients with refractory epilepsy who had previously developed tolerance to clobazam.

Methods

Animal studies

Adult male mice (Tuck No. 1), 25–35 g in weight, were dosed orally, twice daily (07.30 and 19.30 h) for 10 days, with either clobazam (10 mg kg^{-1}) or NDMC (40 mg kg^{-1}) suspended in 1% methylcellulose. In both these experiments control mice received equivalent volumes (5 ml kg^{-1}) of methylcellulose alone on the same schedule. On the first day of each study, and subsequently every 3 days, groups of five experimental and five control animals were randomly selected and 2 h after their morning dose given an i.v. infusion of pentylenetetrazole (PTZ; 10 mg ml^{-1}; 0.3 ml min^{-1}) until a clonic convulsion was elicited. The anticonvulsant protection afforded by each benzodiazepine was calculated as the difference (± s.e. difference) between the mean minimal convulsant doses of PTZ for corresponding experimental and control groups (see Gent *et al.*, 1984). In both studies an extra group of control mice, which had received repeated methylcellulose treatment for 10 days, was given an acute dose of the appropriate benzodiazepine and tested with PTZ. Immediately after their convulsive tests blood samples were taken from those mice which had received a benzodiazepine; plasma levels of clobazam and/or NDMC were analysed by gas liquid chromatography (g.l.c.) and high performance liquid chromatography (h.p.l.c.) respectively. Details of these procedures have been described previously (Gent *et al.*, 1984). All mice were withdrawn from the study following their PTZ infusion. Results were assessed using single classification analysis of variance.

A further study was performed using a higher dose of NDMC (80 mg kg^{-1}) but on this occasion plasma levels of the benzodiazepine were not measured.

Preliminary evaluation of NDMC in refractory epilepsy

The protocol for the study was approved by the Ethics Committee of Leeds General Infirmary and all the patients gave their informed consent.

Nine adult epileptic patients (three male) aged 19–55 years, with poorly controlled complex partial and/or grand mal seizures, who had previously developed tolerance to clobazam, were given NDMC 30 mg daily. Of these patients, seven had been withdrawn from clobazam for a minimum of 2 weeks beforehand (2 weeks–16 months), while the other two (both female) were switched directly from clobazam to NDMC because attempts at withdrawing their clobazam had precipitated seizures. All the patients were continued on other conventional antiepileptic medication, either phenytoin, carbamazepine, phenobarbitone, primidone or sodium valproate (see Table 1). Patients were assessed for between 10–26 weeks during which time seizure records were kept. Plasma concentrations of NDMC were measured at frequent intervals throughout the study and levels of other anti-

Table 1 Details of nine epileptic patients on entry to study

Patient	*Sex*	*Age (years)*	*Additional therapy*	*Holiday from clobazam (weeks)*	*NDMC dose (mg kg^{-1})*
1	F	39	PHT + PR	2	0.48
2	F	40	CBZ + PHT	0	0.58
3	F	19	CBZ	>16	0.61
4	M	55	CBZ + PHT	3	0.35
5	F	32	CBZ	2	0.60
6	F	25	CBZ + PR	0	0.35
7	M	39	CBZ + PR	3	0.41
8	F	32	CBZ + VPA	3	0.64
9	M	38	CBZ + PB + PHT	>16	0.35

CBZ = carbamazepine, PB = phenobarbitone, PHT = phenytoin, PR = primidone, VPA = valproate.

epileptic drugs were measured before and after the onset of NDMC therapy.

Plasma concentrations of NDMC were assayed by reversed phase h.p.l.c. using a modification of the method of Ratnaraj *et al.* (1984). The column was 10 μM Resolve C_{18} Radial Pak (Waters Assoc., Milford, USA) and the mobile phase contained 0.05% ammonia; ether was used as the extraction solvent and diazepam (1.5 μg ml^{-1}) was used as the internal standard.

Results

Animal studies

The acute protection afforded by clobazam (10 mg kg^{-1}; Figure 1a) in mice was significantly reduced from 43.4 ± 2.4 mg kg^{-1} PTZ (mean ± s.e. mean) to 26.5 ± 2.7 mg kg^{-1} PTZ on the fourth day of treatment ($F = 30.58$; $P < 0.001$), but did not change significantly thereafter ($F = 0.21$, $P > 0.25$). Throughout the study, plasma concentrations of clobazam were very low (< 113 ng ml^{-1}) compared with those of NDMC (2300–3920 ng ml^{-1}). Despite the significant reduction in NDMC concentration by the fourth day of treatment ($F = 12.45$; $P < 0.005$), levels on the seventh and tenth days were not significantly different from those on day 1 ($F = 3.47$; $P > 0.05$).

The initial protection afforded by NDMC (40 mg kg^{-1}; Figure 1b) was 35.4 ± 5.1 mg kg^{-1} PTZ. Although this declined to 27.2 ± 2.6 mg kg^{-1} PTZ by the fourth day and remained at that lower level for the rest of the study, no significant change in protection occurred during the 10 days ($F = 1.45$; $P > 0.2$). Despite significant fluctuations in the plasma concentrations of NDMC (1910–3580 ng ml^{-1}; $F = 5.05$; $P < 0.025$), no consistent change was observed.

In a further study, using twice the dose of NDMC (80 mg kg^{-1}; Figure 2) protection fell from 59.2 ± 5.1 to 47.8 ± 3.0 mg kg^{-1} PTZ after 10 days treatment. Once again there was no significant difference between the mean protections on the four test days ($F = 1.78$; $P > 0.1$).

The mean MCD of PTZ in the control groups did not vary significantly during any of these studies ($F \leq 1.27$; $P > 0.25$) ranging from 36.0 ± 1.8 to 40.5 ± 1.8 mg kg^{-1}. In all three studies, the protection afforded by the appropriate dose of benzodiazepine given acutely to control mice on the tenth day was not significantly different from that afforded in naive animals on the first day ($P > 0.7$; Student's *t*-test; Figures 1 and 2). Plasma concentrations of NDMC in such animals were also not significantly different ($P > 0.05$; Student's *t*-test; Figure 1).

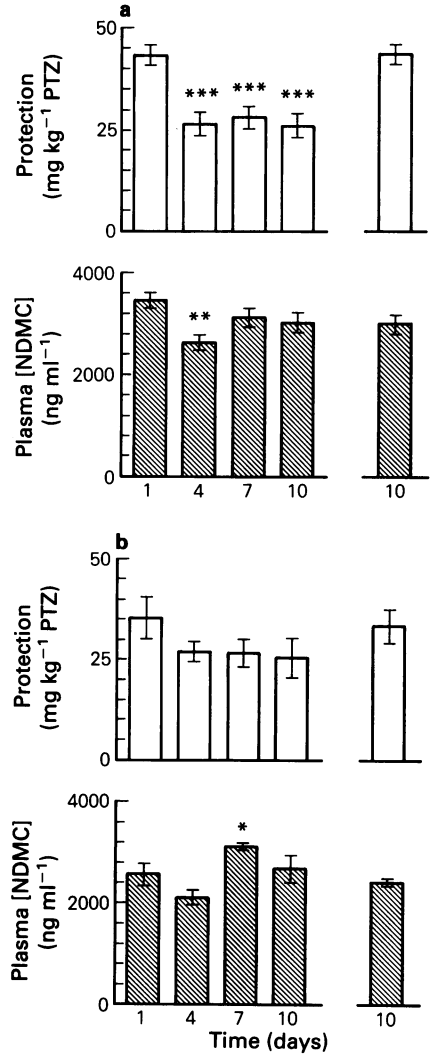

Figure 1 Anticonvulsant protection (open bars) and concomitant plasma concentrations of NDMC (shaded bars) recorded during 10 days oral administration of (a) clobazam (10 mg kg^{-1}; twice daily) and (b) NDMC (40 mg kg^{-1}; twice daily). Values represent mean ± s.e. mean ($n = 5$; 10 mice per estimate of protection). Results for an extra control group given an acute dose of the appropriate benzodiazepine on the tenth day are shown on the right (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with day 1; single classification analysis of variance).

Preliminary evaluation of NDMC in refractory epilepsy

The effects of NDMC on seizure control are shown in Table 2. Eight of the nine patients showed evidence of a favourable response to NDMC. Six of the seven patients who were

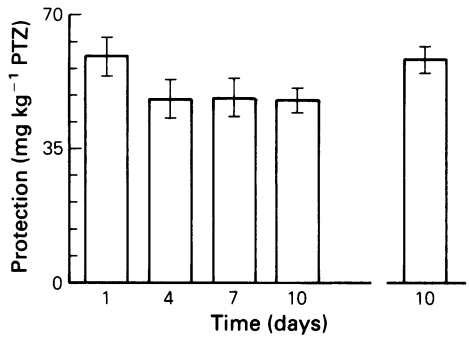

Figure 2 Anticonvulsant protection (mean ± s.e. mean; 10 mice per estimate) afforded by NDMC (80 mg kg^{-1}; twice daily) during 10 days oral administration. The protection afforded by an acute dose of NDMC (80 mg kg^{-1}) in an extra control group on the tenth day is shown on the right. (For statistical analysis see text).

given a 'holiday' from clobazam, including one (No. 8) who had no response to an NDMC placebo while off clobazam, became seizure free for at least 1 month. Four of these subsequently developed a partial tolerance which could be reduced, albeit only transiently, by an increase in the NDMC dose. Only one patient (No. 5) responded poorly to NDMC after a 'holiday' from clobazam; despite an initial reduction in her seizure frequency, very marked tolerance quickly developed which was not improved by an increase in dose. However, the initial response of all seven of these patients to NDMC was similar to, or better than, their initial response to clobazam. Neither of the two patients who were switched directly from clobazam to NDMC became seizure free, but one (No. 2) was improved substantially throughout 24 weeks of NDMC therapy.

Steady state levels of NDMC in these patients were in the range 1000–3000 ng ml^{-1}. One of the patients who was switched directly from clobazam to NDMC had comparable plasma levels of NDMC before and after this change. Of the four patients taking phenytoin concurrently, two (Nos. 1 and 2) showed a substantial increase (~60%) in plasma phenytoin concentration during NDMC therapy, while one (No. 9) experienced a marked fall (~40%) in phenytoin levels. By contrast, carbamazepine levels appeared unchanged in seven patients, and primidone/phenobarbitone levels were unaffected in four patients. One patient made a small reduction in carbamazepine dose, to relieve transient diplopia, before plasma levels could be obtained.

None of the patients found NDMC 30 mg day^{-1} to be sedative, but two of the five patients in whom the dose was increased to 30 mg/60 mg on alternate days complained of sedation.

Table 2 Effect of NDMC on seizure control in nine epileptic patients

Patient	*Pre-NDMC seizure frequency*	*Duration of NDMC therapy (weeks)*	*Seizure control*	
			Seizure free period (weeks)	*Present status*
1	2–3/day	26	5	Partial tolerance† (< 1/week)
*2	5–6/week	24	0	Partial response (< 1/month)
3	3/week	23	4	Partial tolerance† (< 1/week)
4	2/month	16	9	Partial tolerance (~ 1/month)
5	3–4/day for 3–4 days every 2 weeks	15	2	Very limited response† (3–4/day in clusters)
*6	1/week	18	0	Partial response† (< 1/week)
7	2/week	13	5	Partial tolerance† (< 1/week)
8	1–2/week	12	12	Seizure free
9	1–2/week	10	10	Seizure free

* Switched directly from clobazam to NDMC.

† NDMC increased to 30/60 mg on alternate days (after the development of partial tolerance in patients 1, 3 and 7 and only a poor response in patients 5 and 6).

Discussion

In contrast to the significant anticonvulsant tolerance induced in mice by repeated clobazam administration, the protection afforded by two different doses of NDMC did not change significantly during 10 days treatment. Although it seems clear that a fall in protection did occur with NDMC by the fourth day of both studies, this was not the result of a change in metabolism; nor was the tolerance to clobazam caused by a change in the plasma concentration of NDMC, which remained in the same range (2000–4000 ng ml^{-1}) as that produced by NDMC 40 mg kg^{-1}, and which we have previously shown to be responsible for the protection afforded 2 h after clobazam administration (Haigh *et al.*, 1984).

The tentative conclusion from this animal work, that NDMC may produce a lesser tolerance than its parent compound, has found support from a single dose study in the same species, where the development of acute tolerance was shown to require the presence of clobazam (Feely *et al.*, 1986). Moreover, Frey *et al.* (1984) concluded that desmethyldiazepam (given in the form of clorazepate) did not induce anticonvulsant tolerance against PTZ seizures in dogs, whereas repeated treatment with diazepam itself produced a rapid reduction in the anticonvulsant effect. Thus it seems possible that, with the demethylated benzodiazepines at least, there is a difference between the long-term effectiveness of the metabolite when given alone and administration of the parent compound. Reasons for this disparity are not obvious, partly because of the shortage of pharmacodynamic data from longer-term administration of benzodiazepine metabolites. One hypothesis for diazepam was that the accumulation of a longer lasting but less potent metabolite would displace the parent compound from the receptor site through which its actions are mediated (Elsass *et al.*, 1980), but this cannot be supported by the present study as accumulation of NDMC did not occur. Whatever the reason, the possibility that NDMC might induce a lesser tolerance than clobazam in man, as has been suggested for desmethyldiazepam (compared with diazepam) when assessing psychomotor impairment (Elsass *et al.*, 1980), promoted our interest in using NDMC for refractory epilepsy.

We have studied a group of patients who had previously experienced a wide range of initial responses to clobazam, ranging from almost no effect to 7 months remission followed by the development of tolerance. In view of the limitations of such a small, uncontrolled study and bearing in mind that second, or subsequent, responses to clobazam can be disappointing after only a short 'drug holiday', the initial response to NDMC has been very encouraging. Not surprisingly, the least favourable results were seen in the two patients who were switched directly from clobazam to NDMC, but even one of these improved substantially and has continued to respond well for 5 months.

Although it is impossible from an uncontrolled study to predict the effectiveness of NDMC with any certainty, the results appear promising, especially as one patient (No. 8) has remained seizure free for 12 weeks after having had no response to an NDMC placebo for 2 weeks. Despite the marked elevation of plasma phenytoin levels in two of the patients taking this drug concurrently, it seems unlikely that the effect of NDMC on seizure control is the result of interactions with other anticonvulsants. Both these patients had previously been given increasing doses of phenytoin in an attempt to control their seizures and had reached toxic levels without success. Furthermore, one patient (No. 9) experienced a fall in plasma phenytoin concentration during NDMC therapy yet has remained seizure free for 10 weeks. Such bidirectional effects of benzodiazepines on phenytoin levels have been reported previously (Vaijda *et al.*, 1971; Richens, 1977), but there is often no interaction suggesting that in the majority of patients benzodiazepine induced alterations in phenytoin levels are of no great clinical concern (Kutt, 1982). Carbamazepine levels appeared unchanged in patients on concurrent therapy, but concentrations of the 10, 11-epoxide were not measured and an effect on carbamazepine metabolism cannot be excluded; indeed, this might explain the blurred vision reported by one patient. Plasma levels of NDMC (1000–3000 ng ml^{-1}) in patients taking 30 mg daily were in the same range as recorded in patients receiving therapeutic doses (10–30 mg) of clobazam (Callaghan & Goggin, 1984; Allen *et al.*, 1985).

The other major problem with long term benzodiazepine therapy is sedation and psychomotor impairment. Clobazam is preferred to many of the conventional 1,4-benzodiazepines in this respect (Hanks, 1979), but NDMC may have further advantages. NDMC 30 mg was not overtly sedative in any of the patients in contrast to the effects reported by some patients on equivalent or lower doses of clobazam (Feely & Gibson, 1984; Allen *et al.*, 1985). Although a more direct comparison is required, the human responses would support preliminary data from animal studies on the relative psychosedative nature of these two compounds (Fielding & Hoffmann, 1979).

Partial tolerance developed in several patients after 1 month or more of NDMC therapy. However, the length of follow-up in this pilot study, coupled with recent clobazam tolerance in many of the patients, makes any assessment of the tolerance-inducing potential of NDMC compared with clobazam in man inappropriate at this stage. Of the two patients who had a long benzodiazepine-free period before starting NDMC, one (No. 3) never had complete remission with clobazam and the other (No. 9) had a longer complete remission with NDMC (> 10 weeks) than with clobazam (5 weeks). In the patients who developed tolerance the use of an increased, albeit restricted, dose regimen (30/60 mg alternate days) was of variable success, and two of the five in whom this was attempted did complain of sedation; this might have been avoided if smaller dose changes had been possible.

We conclude that NDMC is active in man. An effective dose produces few side-effects, possibly fewer than with clobazam. Since in our animal model tolerance is less with NDMC than with clobazam, we believe that NDMC merits further investigation as an alternative to clobazam for refractory epilepsy.

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